

Phosphorus Distribution in Dairy Manures

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ABSTRACT

The chemical composition of manure P is a key factor determining its potential bioavailability and susceptibility to runoff. The distribution of P forms in 13 dairy manures was investigated with sequential fractionation coupled with orthophosphate-releasing enzymatic hydrolysis. Among the 13 dairy manures, manure total P varied between 4100 and 18 300 mg kg⁻¹ dry matter (DM). Water-extractable P was the largest fraction, with inorganic phosphorus (P_i) accounting for 12 to 44% of manure total P (1400–6800 mg kg⁻¹) and organic phosphorus (P_o) for 2 to 23% (130–1660 mg kg⁻¹), respectively. In the NaHCO₃ fraction, P_i varied between 740 and 4200 mg P kg⁻¹ DM (4–44% of total manure P), and P_o varied between 340 and 1550 mg P kg⁻¹ DM (2–27% of total manure P). In the NaOH fraction, P_i fluctuated around 200 mg P kg⁻¹ DM, and P_o ranged from 130 to 630 mg P kg⁻¹ DM. Of the enzymatically hydrolyzable P_o in the three fractions, phytate-like P dominated, measuring 26 to 605 mg kg⁻¹ DM, whereas monoester P and DNA-like P were relatively low and less variable. Although concentrations of various P forms varied considerably, significant correlations between manure total P and certain P forms were observed. For example, H₂O-extracted P_i was correlated with total manure P ($R^2 = 0.62$), and so was NaOH-extracted P_o ($R^2 = 0.81$). Data also show that the amount of P released by a single extraction with sodium acetate (100 mM, pH 5.0) was equivalent to the sum of P in all three fractions (H₂O-, NaHCO₃-, and NaOH-extractable P). Thus, a single extraction by sodium acetate buffer could provide an efficient evaluation of plant-available P in animal manure, while the sequential fractionation approach provides more detailed characterization of manure P.

ANIMAL MANURE can be a valuable source of P for plant growth. It may also be one of the major sources of the P responsible for increased eutrophication of surface water. The development of best management practices to optimize recycling of manure P and minimize the adverse environmental effects of animal manure application to cropland is of public interest. Research on manure P has often focused on its bioavailability and runoff after application to soils (e.g., Atia and Mallarino, 2002; Chardon et al., 1997; Crouse et al., 2002; Geohring et al., 2001; Griffin et al., 2003; Reddy et al., 1999; Sharpley and Sisak, 1997; Sharpley and Moyer, 2000). Less information is available on characterizing manure P, although the chemical composition of manure P is a key factor affecting P bioavailability and susceptibility to runoff.

A review of the few references that contain information of manure P composition indicates that fractionation by appropriate extractants was used to character-

ize manure P. Early researchers categorized P in swine, poultry, cattle, and sheep manures into phospholipid, inorganic, acid-soluble organic, and residual forms (Barnett, 1994a, 1994b; McAuliffe and Peech, 1949; Peperzak et al., 1959). These reports show that P_i constitutes an important proportion, followed in descending order of importance by residual P, acid-soluble organic P, and small amounts of phospholipids. These reports also indicate that concentrations of these manure P forms were affected by many plant and animal factors, such as dietary content and animal species. Barnett (1994a) further found that total, acid-soluble organic, and lipid P varied more, and inorganic and residual P varied less in ruminant than nonruminant fecal materials. Although useful information on manure P availability for plants could be estimated, this approach may be inadequate to address the concern of dissolution and runoff loss of manure P (Dou et al., 2000).

More recently, a sequential fractionation strategy originally developed by Hedley et al. (1982) for soil P characterization has been adapted and modified for characterizing manure P (Leinweber et al., 1997; Dou et al., 2000; Sharpley and Moyer, 2000; He and Honeycutt, 2001). In this approach, manure P is classified as H₂O- or resin-, NaHCO₃-, NaOH-, and HCl- or H₂SO₄-extracted P as well as residual P. Organic P in each extractant is estimated by the difference between total phosphorus (P_t) and P_i. The majority of manure P was found in the first three fractions in swine, dairy, and poultry manures by three research groups in the United States (Dou et al., 2000; He and Honeycutt, 2001; Sharpley and Moyer, 2000). In Germany (Leinweber et al., 1997), however, more than 50% of P_i was reported in acid and residual fractions for chicken and liquid swine manures. The authors partially attributed the observation to insoluble mineral phases in the manures. As pointed out by Sharpley and Moyer (2000), although the fractionation approach is cheap and can provide a rapid estimate of P solubilities and labilities, interpreting the chemical composition of P_i and P_o from chemical fractionation can be risky due to the intrinsic obscurity of the extractants used and the different physicochemical properties of manure and soil (He et al., 2003).

Phosphorus-31 nuclear magnetic resonance (NMR) spectroscopy can provide information on P species in animal manure. Leinweber et al. (1997) demonstrated with ³¹P NMR spectra of NaOH extracts that chicken manure orthophosphate accounted for more than 50% of P_i, with lesser amounts of monoester and diester P. In swine manure, about 50% of P_i was monoester P, with about equal amounts of P_i and diester P. One advantage of this NMR method is its ability to account for all organic P directly in an extract sample. However,

Abbreviations: DM, dry matter; P_i, inorganic phosphorus; P_o, organic phosphorus; P_t, total phosphorus.

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the alkaline conditions required for NMR running can result in hydrolysis of some labile organic P species (Leinweber et al., 1997). Furthermore, the ecological significance of the ^{31}P NMR-visible P species (i.e., dissolution and plant availability) was not clear, and required additional studies (Leinweber et al., 1997).

Phosphatase hydrolysis provides another approach for classifying manure P. We designed a phosphatase hydrolysis method to characterize swine and cattle manure P (He and Honeycutt, 2001) and further improved the method (He et al., 2004). In this enzymatic approach, P_o in H_2O -, NaHCO_3 -, and NaOH -extracted fractions of manure P were incubated with acid phosphatase from potato, acid phosphatases from potato and wheat germ, and both phosphatases plus nuclease P1, separately, at pH 5.0. The P released differentially by these enzymes was designated as simple monoester P, phytate (hexaphosphate)-like P, and DNA-like P, respectively. Where most studies report only total P_o in sequentially extracted fractions, the enzymatic hydrolysis is able to separate P_o into enzymatically hydrolyzable organic P species and enzymatically nonhydrolyzable P. The former is considered labile or bioavailable P, the latter recalcitrant P. These two types of organic P show different dynamic patterns after incorporation to soils (He et al., unpublished data, 2004), suggesting the importance of distinguishing the hydrolyzable P_o forms.

The primary objective of the present work was to improve the understanding of manure P chemistry by characterizing 13 dairy manures with the sequential fractionation coupled with phosphatase hydrolysis. We also evaluated the relationship of P extracted by single sodium acetate buffer (100 mM, pH 5.0) with the H_2O -, NaHCO_3 -, and NaOH -extractable P in the sequential fractionation because this single extraction may offer a rapid and efficient evaluation of plant-available P in animal manure.

MATERIALS AND METHODS

Manures and Fractionation

Thirteen dairy manure samples were obtained from local commercial dairy farms (via the University of Maine Analytical Laboratory), representing a range in storage types and bedding options. Each sample was homogenized, freeze-dried, ground to pass 2 mm, and stored at -20°C until use. Total N

was measured by Kjeldahl digestion. Total P was measured by dry combustion, followed by digestion in 0.5 M H_2SO_4 and inductively coupled plasma emission spectroscopy. Selected properties of these manures are listed in Table 1.

The sequential fractionation scheme described in He et al. (2004) was used in this study. Each sample (0.25 g of manure) was sequentially extracted in 25 mL of deionized H_2O for 2 h, 0.5 M NaHCO_3 for 16 h, and 0.1 M NaOH for 16 h. Extractions were performed at 22°C on an orbital shaker (250 rpm). Samples were then centrifuged at $23\,700 \times g$ for 30 min at 4°C and the supernatant was decanted and filtered through a 0.45- μm filter. Manure samples (0.25 g each) were also separately extracted by 25 mL of 100 mM Na acetate buffer (pH 5.0) for 1 h, then 10 mL of the buffer for 1 h. Both extracts by the buffer were combined. Experiments were performed in triplicate.

Enzymes and Enzymatic Incubation

Acid phosphatases (EC 3.1.3.2), type I from wheat germ (0.5 U mg^{-1} solid) and type IV-S from potato (5.3 U mg^{-1} solid), and nuclease P1 (EC 3.1.30.1) from *Penicillium citrinum* (355 U mg^{-1} solid), were purchased from Sigma (St. Louis, MO). One unit (U) of enzyme activity was defined as liberation of 1.0 μmol of relevant product from appropriate substrates at optimal incubation conditions based on the supplier's information.

All enzymatic incubations were performed at 37°C for 1 h. The NaHCO_3 and NaOH fractions were neutralized to pH 5.0 by slow addition of 2.5 or 8 M acetic acid. EDTA (1 mM final concentration) was added to the NaOH fraction to prevent phosphorus compounds from precipitating during pH adjustment. The incubation mixtures contained enzymes (acid phosphatases 0.25, and nuclease P1 2 U mL^{-1} mixture) and 100 mM Na acetate (pH 5.0) (He et al., 2004). Controls were included whereby either the enzyme or samples (substrates) were omitted.

Phosphorus Determination

Orthophosphate (i.e., inorganic P, P_i) was assayed by a molybdate blue method modified by Dick and Tabatabai (1977), with total assay volume reduced to 1 mL. It is worth noting that this method is developed for accurate determination of P_i , whereas other molybdate blue methods determine a loosely defined "molybdate-reactive P" which may include some labile P_o and condensed P_i (Dick and Tabatabai, 1977; Haygarth and Sharpley, 2000). Total P was determined in the same way after H_2SO_4 - H_2O_2 digestion and adjustment to pH 5. Organic P was estimated as the difference between total P and P_i . Enzyme-released P was calculated as the difference between P_i contents determined in the presence and absence of the enzyme(s). Simple monoester P was determined by

Table 1. Characteristics of dairy manure used in this study.

Sample	Dry matter	NH ₄ -N	Organic N	Total P	EC†	pH†
	%	mg kg ⁻¹ DM‡			dS m ⁻¹	
27	22.9	5 700	14 800	4 100	3.7	7.4
29-1	14.6	19 900	17 100	5 600	12.0	8.2
430-1	3.5	45 700	40 000	18 300	10.3	6.1
725	7.2	18 100	20 800	12 700	10.1	6.5
727	12.3	1 600	22 800	13 100	3.3	6.6
733	4.2	35 700	26 200	9 600	14.9	7.5
1067	8.1	17 300	16 000	5 400	5.5	7.1
RK-2	8.5	15 300	21 200	8 200	8.1	9.1
RK-3	11.7	16 200	17 900	7 700	8.9	7.6
RK-4	14.1	3 500	12 100	9 200	4.8	8.1
RK-6	10.6	13 200	18 900	8 500	6.2	8.5
45	20.6	5 800	12 100	7 500	2.8	7.1
RK-1	13.7	16 800	17 500	8 800	11.1	6.5

† Electric conductivity (EC) and pH were determined on a 6:1 water (mL) to dry manure (g) ratio.

‡ DM, dry matter.

the difference in P contents determined in the presence and absence of potato phosphatase. Phytate-like P was calculated as potato and wheat germ phosphatases–released P minus potato phosphatase–released P. The DNA-like P was calculated from P released by all three enzymes minus potato- and wheat germ phosphatases–released P (He et al., 2004).

RESULTS AND DISCUSSION

Dry Matter and Total Phosphorus Contents

Among the 13 dairy manures, the dry matter (DM) content varied between 4.2 and 22.9% of fresh manure weight, and total manure P between 4100 and 18 300 mg kg⁻¹ DM (Table 1). The average DM content was 11.7% with standard deviation (SD) of 5.7%. Average total manure P was 9130 mg kg⁻¹ DM with a SD of 3770 mg kg⁻¹ DM. The wide range and variability of these parameters among the 13 samples reflect a combined differences in feed ingredients, animal characteristics and production levels, and other management factors such as type of bedding materials in use and manure storage conditions. Barnett (1994a) reported that in fresh uncontaminated feces of 15 dairy herds on commercial farms, DM varied from 13.5 to 16.2% with an average of 14.3% and SD of 9.0%, whereas P_i varied from 6000 to 16 000 mg kg⁻¹ DM with an average of 9300 and SD of 3000. Sharpley and Moyer (2000) reported that in 24 dairy manures collected over two years, DM varied from 25.6 to 35.0% with an average of 30.1% and SD of 2.4%, whereas total manure P varied from 1500 to 7800 mg kg⁻¹ DM with an average of 3500 and SD of 2000. Thus, values of the data obtained in this work were similar to those reported by Barnett (1994a), but considerably less than the data reported by Sharpley and Moyer (2000). This may be because the latter data were obtained from an experimental facility at a single location, which reduced the variations caused by the different raising practices on different farms.

Total manure P was negatively related to the DM content of the 13 manures (Fig. 1), suggesting that the quality of animal manure in terms of nutrient P decreased with greater percentages of DM in the 13 samples. We, however, did not have adequate information to explain the observation. Any conclusive correlation

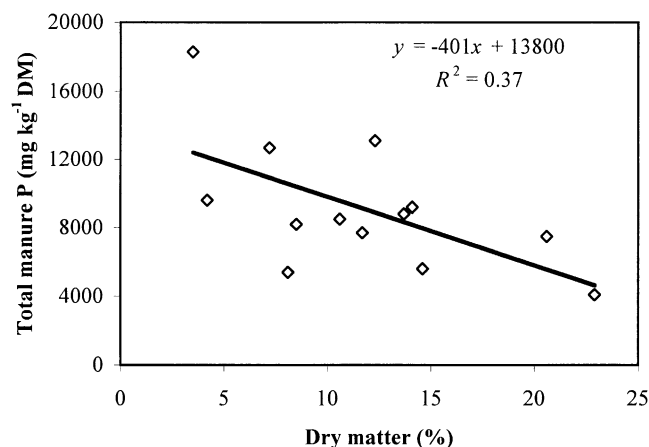


Fig. 1. Relationship of total manure P with dry matter content of dairy manure.

and explanation can only be established after more extensive research.

Phosphorus Forms in Sequential Fractions

Previous observations by Dou et al. (2000, 2002), He and Honeycutt (2001), and Sharpley and Moyer (2000) indicate that most of the P in dairy manure was soluble in H₂O, NaHCO₃, and NaOH, leaving only a small amount of HCl-extractable P. In this study, we therefore omitted the HCl extraction step in the sequential extraction of dairy manures.

A large portion of total dairy manure P was extracted by H₂O. The extracted P_i varied between 1000 and 6800 mg P kg⁻¹ DM (12–44% of total manure P), and P_o varied between 130 and 1700 mg P kg⁻¹ DM (2–23% of total manure P) in the 13 manure samples (Fig. 2A). In the H₂O fraction, P_i was more highly correlated ($r^2 = 0.62$) to total manure P than P_o ($r^2 = 0.24$). The slope of the linear regression equation for H₂O-extractable P_i indicates that each unit increase of manure total P would bring a 0.32-unit increase of water-soluble P_i. In the NaHCO₃ fraction, P_i varied between 740 and 4200 mg P kg⁻¹ DM (4–44% of total manure P), and P_o varied

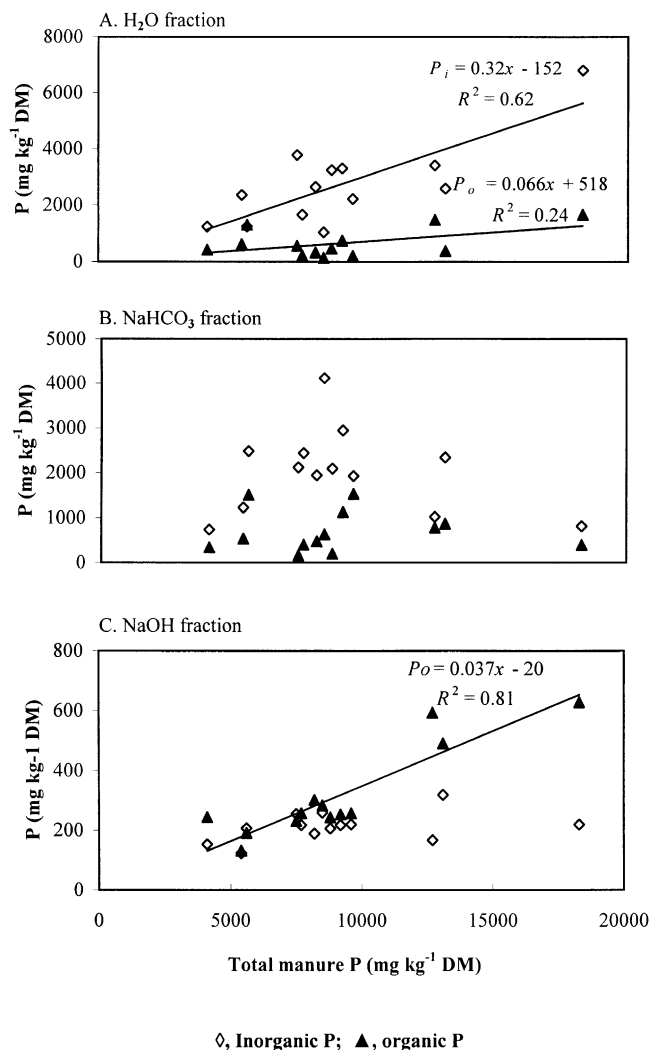


Fig. 2. Distribution of inorganic and organic P in sequential fractions of dairy manure.

between 340 and 1550 mg P kg⁻¹ DM (2–27% of total manure P). However, no apparent correlation of either P_i or P_o to total manure P was observed (Fig. 2B). More of the total manure P was present as P_o than P_i in the NaOH fraction (Fig. 2C). Inorganic P fluctuated around 200 mg P kg⁻¹ DM (1–4% of total manure P). In contrast to the H₂O fraction, P_o (2–6% of total manure P), not P_i, was positively correlated to total manure P ($r^2 = 0.81$). The high amount of P_i in the H₂O fraction, and its positive correlation with total manure P, may reflect the mineral composition of and oversupply of feedstuff P (Dou et al., 2002). Lack of significant correlation for either P_i or P_o in the NaHCO₃ fraction may reflect the intermediate ability of NaHCO₃ to extract P. In other words, much of the readily soluble P was already extracted by H₂O; the majority of tight-bound P was only extractable by a strong extractant, such as NaOH.

Relative amounts of P in sequential fractions of these 13 dairy manures were consistent with our previous observation (He and Honeycutt, 2001). In a representative dairy manure, Sharpley and Moyer (2000) observed that P_i comprised 51, 9, and 2% of P_t in the H₂O, NaHCO₃, and NaOH fractions, respectively. The percentage of P_o was 12, 2, and 11% of P_t in the three fractions. Dou et al. (2000) reported that H₂O-, NaHCO₃-, and NaOH-extracted P were 70, 14, and 6% of total manure P, respectively. Whereas the general trend of P distribution in the three fractions is similar, differences in specific values might arise from the specific extraction procedures adapted, such as shortened extraction times. Dou et al. (2002) observed that increasing dietary P concentrations resulted in a greater amount of P in the H₂O fraction of dairy manure, whereas P concentrations in other fractions remained low. Their observation could be an explanation for the positive correlation between H₂O-extractable P_i and total manure P observed in this study. The lability of H₂O-extracted P_o made it poorly related to total manure P.

Distribution of Hydrolyzable Organic Phosphorus in Sequential Fractions

We further examined the distribution of hydrolyzable P_o forms in the three sequential fractions of 13 dairy manures with the enzymatic approach of phosphatase hydrolysis (He et al., 2004). The quantity of simple monoester P ranged from none to about 100 mg kg⁻¹ DM (2% of total manure P) in both H₂O and NaHCO₃ fractions, and from 25 to 100 mg kg⁻¹ DM in the NaOH fraction (Fig. 3). The proportions of DNA-like P were in the same range as that of simple monoester P with exceptions of 150 mg kg⁻¹ DM in one H₂O fraction and 200 mg kg⁻¹ DM in one NaHCO₃ fraction (Fig. 3). The relatively stable and low amounts of these two types of P may be attributed to the hydrolysis catalyzed by phosphatase and phosphodiesterase activity existing in dairy manure (Dick and Tabatabai, 1984).

Phytate-like P (up to 5% of total manure P in each fraction) was present as the major hydrolyzable P_o form in all three fractions. The concentration of phytate-like P in the H₂O fraction increased along with total manure P (Fig. 3A). Amounts of phytate-like P in NaHCO₃ and

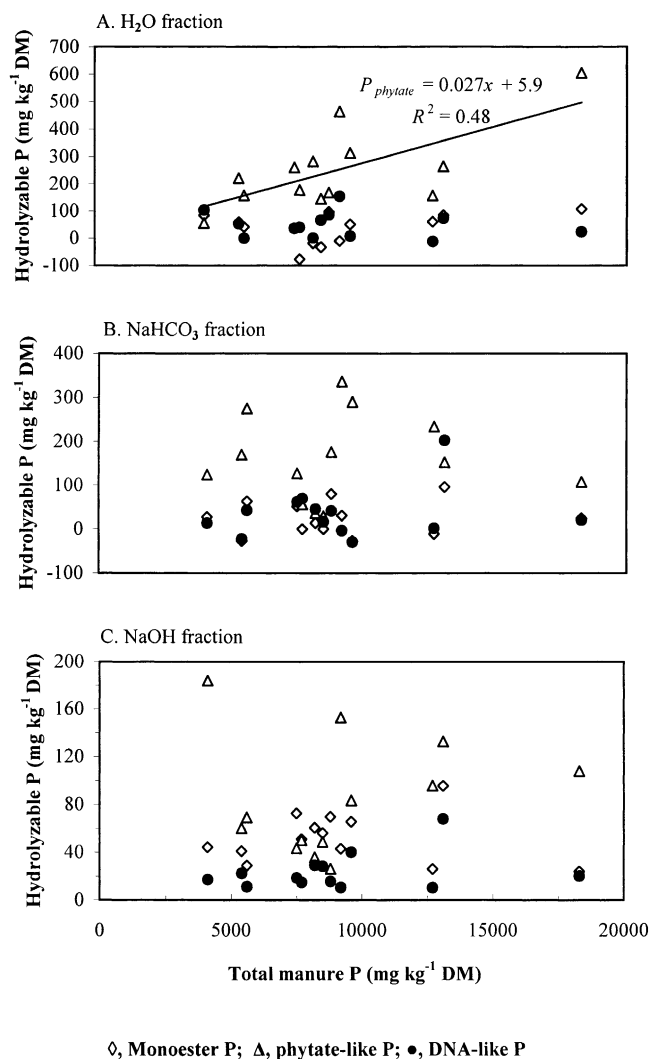
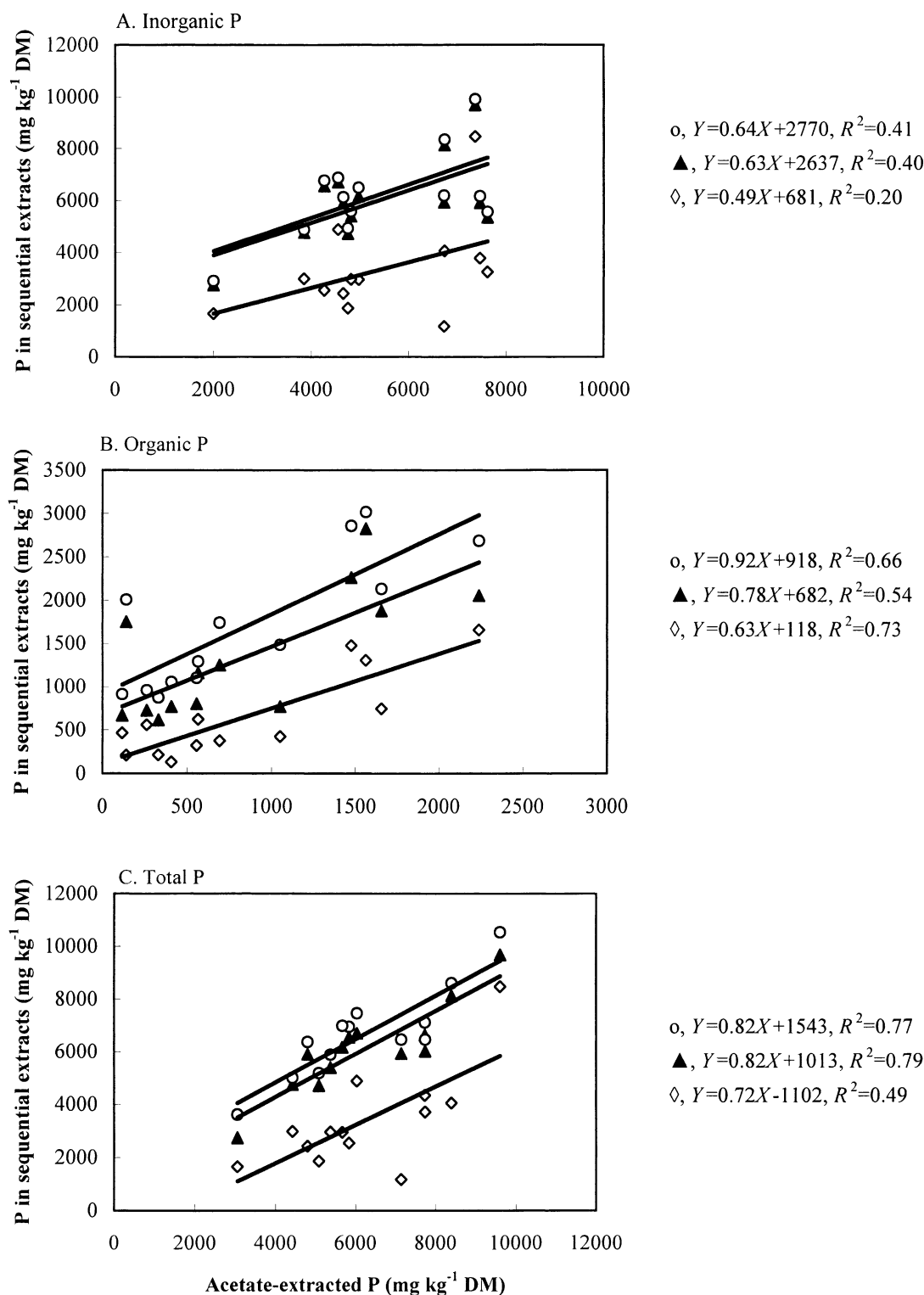


Fig. 3. Distribution of enzymatically hydrolyzable organic P in sequential fractions of dairy manure.

NaOH fractions were not related to changes in total manure P (Fig. 3B and 3C). In contrast, phytate-like P in NaHCO₃ and NaOH fractions was better related to organic P in each fraction ($P_{\text{phytate}} = 0.24P_o - 53$, $R^2 = 0.52$, and $P_{\text{phytate}} = 0.15P_o + 36$, $R^2 = 0.41$, respectively). These data indicate that phytate-like P in the three fractions might have originated from different sources. The P_o in manures is in part directly from the feedstuff, in part excreted by the animal, and in part synthesized by the microorganisms in and outside of the digestive tract (Peperzak et al., 1959). Even soluble P_i added into the feedstuff can appear in the P_o fraction as shown by isotope-labeled P uptake experiments (McAuliffe and Peech, 1949). We hypothesize that phytate-like P present in the H₂O fraction could be undigested phytate from feedstuffs, thus exhibiting a closer relationship with quantity of total manure P. The phytate-like P in other two fractions might be the result of microbial activities in the manure before and after excretion.

Phosphorus Extracted by Sodium Acetate Buffer

The sequential fractionation approach provided comprehensive information on manure P characteristics.



◊, H₂O fraction; ▲, H₂O and NaHCO₃ fractions; o, H₂O, NaHCO₃, and NaOH fractions

Fig. 4. Relationships of sodium acetate buffer (100 mM, pH 5.0) extracted P with H₂O-extractable P, summed H₂O- and NaHCO₃-extractable P, or summed H₂O-, NaHCO₃-, and NaOH-extractable P in the sequential fractionation.

However, the procedure takes considerable time. Thus, single H₂O-extracted P has been proposed to serve as an indicator of potential P runoff (Dou et al., 2002;

Kleinman et al., 2002; Sharpley and Moyer, 2000). However, H₂O-extractable P is not the only P in manure available for plant growth (Atia and Mallarino, 2002).

Morgan (1.24 M sodium acetate buffer, pH 4.8) or modified Morgan (0.62 M NH_4OH + 1.25 M acetic acid, pH 4.8) P has been used in Maine as a soil P indicator (Griffin et al., 2003). Therefore, we tested sodium acetate buffer (100 mM, pH 5.0) as a single extractant for plant-available P in manure. We selected pH 5 because it was close to that for Morgan or modified Morgan P and was the optimal pH required for enzymatic hydrolysis. In the 13 dairy manures, the amounts of both P_i and P_o extracted by sodium acetate buffer were greater than those extracted by H_2O alone, ranging from 2000 to 7400 mg P kg^{-1} DM. The concentration of P_o fluctuated around 1000 mg P kg^{-1} DM, with a range from 140 to 2200 mg P kg^{-1} DM. The acetate buffer-extracted P revealed interesting relationships with P forms in sequential fractions. Buffer-extracted P_i was more strongly correlated with the sum of P_i in the H_2O and NaHCO_3 fractions ($r^2 = 0.41$) than P_i in the H_2O fractions ($r^2 = 0.20$) (Fig. 4A). Inclusion of NaOH-extracted P_i in the sum increased the y intercept only slightly because the amount of P_i extracted by NaOH in the sequential fractionation was relatively small at around 200 mg P kg^{-1} DM in the 13 manures (Fig. 2A). In contrast, the buffer-extracted P_o was better correlated to P_o in the H_2O fraction ($r^2 = 0.73$) than the sum of P_o in the two fractions ($r^2 = 0.54$) (Fig. 4B). The acetate buffer-extracted P_i was correlated to the sum of P_i in the H_2O and NaHCO_3 fractions with a greater value of 0.89 (Fig. 4C) than either P_i or P_o alone. This observation might reflect the fact that different degrees of P_o hydrolysis occurred during acetate and sequential extractions. Total P extracted by the acetate buffer had an average of 6221 mg P kg^{-1} DM with SD of 1811. Total P in both H_2O and NaHCO_3 fractions had an average of 6104 mg P kg^{-1} DM with SD of 1668. The average of total P in all three fractions was 6669 mg P kg^{-1} DM with SD of 1701. These data indicate that the amount of P extracted by single sodium acetate buffer (100 mM, pH 5.0) from dairy manure was equal to the summed amount of P extracted by H_2O , NaHCO_3 , and NaOH in the sequential fractionation. We were not able to conclude whether NaOH-extractable P was really extracted by sodium acetate buffer due to the low amount of P extracted by NaOH in the sequential fractions. The property of sodium acetate buffer may imply an exclusion of NaOH-extractable P in the acetate pool. In spite of this obscurity, the correlation between acetate-extracted P and the sequentially extracted P suggested that the single acetate-extracted P covered all plant-available P that was identified in the three sequential fractions based on the assumption that H_2O - and NaHCO_3 -extracted P are labile, and NaOH-extracted P is somewhat labile (Hedley et al., 1982; Cross and Schlesinger, 1995). Therefore, a single extraction by sodium acetate buffer could provide a rapid and efficient evaluation of plant-available P in animal manure, while the sequential fractionation approach provides more detailed characterization of manure P.

CONCLUSIONS

Concentrations and composition of manure P often varied considerably. Among the 13 dairy manures evalu-

ated in the present study, manure total P varied between 4100 and 18 300 mg kg^{-1} DM with an average of 9130 mg kg^{-1} DM. It is noticeable, however, that total manure P was negatively related to the DM content in the 13 manures. A large portion of dairy manure P was extracted by H_2O . In the H_2O fraction, P_i ($r^2 = 0.62$) is better correlated to total manure P than P_o ($r^2 = 0.24$). No apparent correlation of either P_i or P_o to total manure P was observed in the NaHCO_3 fraction. In the NaOH fraction, the amount of P_o is greater than that of P_i , and P_o , not P_i , was positively correlated to total manure P ($r^2 = 0.81$). Phytate-like P was present as the major hydrolyzable P_o in all three fractions (26–605 mg kg^{-1} DM). The concentration of phytate-like P in the H_2O fraction increased with increasing total manure P. Concentrations of simple monoester P and DNA-like P were low in all three fractions.

The average P_i extracted by single sodium acetate buffer (100 mM, pH 5.0) was equal to the summed amount of H_2O -, NaHCO_3 -, and NaOH-extractable labile P in the sequential fractionation. Thus, single extraction by sodium acetate buffer could be used for fast evaluation of plant-available P in animal manure with less workload than application of the sequential fractionation approach.

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